Biochemical Identification of Feces of Nine Neotropical Carnivores

by

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ABSTRACT

The bile-acid patterns is used as a method of identifying carnivore feces to species. We assess the presence of bile acids in feces of some southern Neotropical carnivores to identify feces collected in the field, using TCL method. The patterns for. Dusicyon griseus and D. culpaeus was determine. The presence of chenodeoxycholic acid, hyodeoxycholic acid, one identified acid in the last one permits separated one species to the other. A blind proof was conducted. According to this, culpeo and chico grey fox feces could be correctly identified in 85 % of the cases. Our analysis provides a mean of avoiding gross errors which might have been incorporated into studies of those species.

The use of bile-acid patterns as a method of identifying carnivore feces to species was the result of attempts to develop non destructive techniques for assessments of population size and studies of diets of endangered species (Major et al., 1980; Johnson et al., 1981).

Feces of carnivores usually are similar in shape and size. Attempts have been made to identify species based on diameters of feces, but the size ranges of feces overlap too much to be useful as a distinctive character (Weaver and Fritz, 1979, for coyotes (Canis latrans) and wolves (Canis lupus); Daner and Dodd, 1982, for coyotes and grey foxes (Urocyon cynereoargenteus)). Instead, the study of distributions of bile acids in feces was a satisfactory technique to distinguish different species of carnivores (Aldred, 1980; Clinite, 1981; Johnson and Aldred, 1981; Johnson et al., 1981, 1984; Major et al., 1980).

For neotropical carnivores, no studies on identification of feces based either on diameter or bile-acid patterns are available. Herein, we assess the presence of bile acids in feces of some southern Neotropical carnivores to identify feces collected in the field.

METHODS

The basic procedures used to determine composition of bile acids

are thin-layer chromatography (Kritchevsky et al., 1963) and gas-liquid chromatography (Young, 1978). We applied the first methodology, which was proposed by Johnson et al. (1981) to identify 18 carnivore species from North America at the lowest possible cost. We followed the steps suggested by Major et al. (1980) and Johnson et al. (1981).

The following are the numbers of individuals of each of the nine species studied: eleven culpeo foxes (Dusicyon culpaeus), ten chico grey foxes (Dusicyon griseus), four Pampa's foxes (Dusicyon gymnocercus), two crab-eating foxes (Cerdocyon thous), two Chilean foxes (Dusicyon fulvipes), ten domestic dogs (Canis familiaris), two Geoffroyi's cats (Felis geoffroyi), two Pampa's cats (Felis colocolo), and two Molina's skunks (Conepatus chinga). Feces were collected at the Auca Cuyín Zoo (Piedra del Aguila, Neuquén Province, Argentina), Buenos Aires Zoo (Buenos Aires, Argentina), and Chilean fox feces were sent to us by Lic. Jaime Jimenez of Universidad Católica de Chile.

Chromatography runs of feces were conducted with a known mixture of bile acids (equal parts of chenodeoxycholic, cholic, deoxycholic, hyocholic, hyodeoxycholic, litholic and ursocholic acids) and cholesterol. These are the most common acids reported from carnivore feces (Johnson et al, 1981, 1984, 1986; Major et al. 1980). Cholesterol was added as a reference because any spot which runs faster than it is not a bile acid. Comparison with these

controls allowed identification of the different bile acids in extracts from feces of each species. This comparison was made considering spot color and Rf values (distances travelled by a spot divided by the distance travelled by the solvent front). Because variables such as humidity and temperature influence Rf values and color, standard bile acids should be used for reference in each experiment (Haslewood, 1967). To evaluate the reliability of the identification a blind proof was conducted using feces from 10 culpeo and 10 chico gray foxes. The other species were not included in this proof due to the small sample size.

RESULTS

The mean and standard deviation of the RF for each bile acid was calculated for each species with at least ten individuals (Table 1). There were no differences in bile acid composition among individuals from the same species. Thus, Table 2 was elaborated pooling the results of all samples from each species. Table 2 shows the presence of each of the five known bile acids, cholesterol, and of six unknown bile acids for the nine carnivore species studied. Some organic compounds, such as pigments, were observed, but as their Rf values were always larger than that of cholesterol they were disregarded.

Lithocholic and deoxycholic acids were present in all species,

hyocholic acid appeared only in the culpeo fox while chenodeoxycholic acid appeared in both cats and in the culpeo fox. Ursocholic acid was not detected in any species, cholic acid was not found in the skunk and cats, while hyodeoxycholic acid was absent in the crab-eating fox, Pampa's fox and Chilean fox. The remaining 6 spots were considered unidentified bile acids because their Rf values were lower than cholesterol Rf.

D. fulvipes presented few bile acid spots (Table 2); this could be attributed to the fact that a very small sample was available (less than the one gram recommended for this method), so that extracts were probably too diluted. Presence of spots 5 (unidentified acid), 7 (chenodeoxycholic acid), and 11 (hyodeoxycholic acid), and absence of spot 3 proved to be important diagnostic characters in the identification of culpeo fox feces (Tables 1 and 2). Inversely, presence of spot 3 and absence of spots 5, 7, and 11 identified chico grey fox feces. All four spots (3, 5, 7 and 11) were present in dog feces. Spot 14 (cholic acid) was taken as a distinguishing feature of canid feces, because skunks and cats lacked this acid (Table 2).

Eighty five percent (17/20) of the samples were identified correctly in the blind proof. Only three feces were misidentified: two were identified as chico grey fox when they were from culpeo fox. The third one could not be matched with culpeo nor with chico grey fox, when it was actually from the latter.

DISCUSSION AND CONCLUSION

Our dog sample presented a similar pattern to the one showed by Major et al. (1980): cholic, deoxycholic, hyodeoxycholic, and lithocholic acids were present. Deoxycholic and lithocholic acids were present in all the species studied here, as they were in carnivores species studied by Major et al. (1980) in North America. Cholic acid appeared only in canid feces in our study, although it was found in several non-canid carnivores like bobcat (Felis rufus), mountain lion (Felis concolor), domestic cat (Felis catus), mink (Mustela vison), raccoon (Procyon lotor), and stripped skunk (Mephitis mephitis), and marsupials like the Virginia opossum (Didelphis virginiana) (Major et al., 1980; Johnson et al. 1981).

Unfortunately, not enough samples were available to allow reliable identification of bile acid patterns of all species involved in this study. However, it was possible to determine the bile acid patterns of culpeo fox and chico gray fox, and to distinguish them from dog feces. These three species have overlapping distributions in southern South America (Medel and Jaksic, 1988), and thus our findings could be useful for future food habits studies. Only culpeo fox feces had hyocholic (Rf=0.32, color = yellow) and chenodeoxycholic acids (Rf=0.41, color = green), dogs had spot 5 (Rf=0.55; color = fluorescent under UV light), while chico grey fox did not have any of these spots (Table 1).

Field identification of feces is difficult especially when several species of similar body dimensions coexists in one area. Bile acids are very stable: they have been detected in 2000-year-old coprolites found in dry habitats (Lin et al. 1978). Therefore, the study of patterns of bile acids in feces could be a good alternative for their identification. Although rain could affect this identification (Johnson et al., 1984), it would be feasible in feces that are not too old or are found in dry habitats. According to our blind proof, culpeo and chico grey fox feces could be correctly identified in 85 % of the cases. This appears more reliable than field identifications were color, shape, smell, and size of the feces are used. Our analysis provides a mean of avoiding gross errors which might have been incorporated into studies of those species (Medel and Jaksic, 1988), which are distributed in dry areas of South America where the bile-acid technique could be succesfully applied.

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Table 1. Mean Rf value and standard deviation (in parenthesis) of bile acid spots in thin-layer chromatographies of feces of culpeo and chico gray fox and dogs (N = 20 chromatography runs for each species).

Spot	ZC	ZG	P	M
1	0.83 (0.01)	0.84 (0.01)	0.84 (0.03)	col
2	0.69 (0.05)	0.69 (0.04)	0.68 (0.02)	lit
3		0.63 (0.01)	0.52 (0.01)	
4	0.58 (0.01)	0.59 (0.01)	0.59 (0.01)	
5	0.55 (0.02)		0.54 (0.03)	
6	0.48 (0.01)	0.49 (0.01)	0.47 (0.01)	deo
7	0.41 (0.01)			que
8				urs
9				
10				
11	0.32 (0.01)			hio
12				
13	0.28 (<0.01)	0.27 (<0.01)	0.29 (<0.01)	hid
14	0.14 (<0.01)	0.13 (<0.01)	0.13 (<0.01)	coi

Spot 1 corresponds to the substance with the highest Rf value (closest to the solvent front). ZC: culpeo fox; ZG: chico gray fox; P: dog; M: mixture of known organic compounds; Rf: distance travelled by a spot divided by the distance travelled by the solvent;; col: cholesterol; coi: cholic acid; deo: deoxycholic

acid; hio: hyocholic acid; hid: hyodeoxycholic acid; que: chenodeoxycholic acid; urs: ursocholic acid.

Table 2. Presence of spots corresponding to organic compounds obtained by thin layer chromatography of feces of nine Patagonian carnivore species.

Spot	ZC	ZG	ZP	ZN	ZH	P	ZO	GP	GM	M	Rf	CO
1	*	*	*	*	*	*	*	*	*	col	0.84	re
2	*	*	*	*	*	*	*	*	*	lit	0.69	br
3		*	*	*		*					0.62	ye
4	*	*	*	*		*	*	*	*		0.59	vi
5	*					*					0.55	fl
6	*	*	*	*	*	*	*	*	*		0.48	ye
7	*							*	*		0.41	gr
8											0.38	br
9							*	*	*		0.35	gr
10					*						0.33	vi
11	*									hio	0.32	ye
12			*								0.31	re
13	*	*				*	*	*	*	hid	0.29	fl
14	*	**	**	*	*	**				coi	0.14	ye

Spot 1 corresponds to the substance with the highest Rf value (closest to the solvent front). ZC: culpeo fox; ZG: chico gray fox; ZP: Pampa's fox; ZN: crab-eating fox; ZH: Chilean fox; P: dog; ZO: skunk; GP: Pampa's cat; GM: Geoffroyi's cat; M: mixture of known organic compounds; Rf: distance travelled by a spot divided by the distance travelled by the solvent; CO: spot color using sulphuric acid as developer; * : clearly visible spot; **: slightly visible spot; col: cholesterol; coi: cholic acid; deo: deoxycholic acid; hio: hyocholic acid; hid: hyodeoxycholic acid; que: chenodeoxycholic acid; urs: ursocholic acid; br: brown; fl: fluorescence under shortwave UV light; gr: green; re: red; vi: violet; ye: yellow.